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Manipulating the N release from ^{15}N-labelled celery residues by using straw and vinasses in Flanders (Belgium)

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Abstract

The effect of straw and vinasses on the N mineralization–immobilization turnover of celery residues was investigated in a field experiment in Flanders (Belgium). The field was laid out in raised beds on a loamy sand soil. At the start of the experiment, ^{15}N-labelled celery residues (4 t dry matter (DM) ha^{-1}) were mixed with straw (12 t DM ha^{-1}) in order to immobilize the released celery-N followed by an incorporation of vinasses (4 t DM ha^{-1}) after 200 days aiming to remineralize the immobilized N. Total N, mineral N and their ^{15}N enrichments as well as microbial biomass N were determined at regular time intervals. During the first 62 days after the incorporation, straw immobilized the celery derived ^{15}N in the microbial biomass and reduced the total celery-^{15}N losses from the top 25 cm by 38%. However, after 62 days, the N immobilization ceased due to low temperatures (<10 °C) in the raised beds, and was followed by natural remineralization of immobilized celery-^{15}N (without addition of vinasses) at a moment when the risk of nitrate leaching was still high. Hence, straw was not able to reduce the celery-N losses during the complete winter period. The addition of vinasses in spring caused no real positive priming effect, although it did increase the amount of remineralized celery-^{15}N (7.6% of celery derived ^{15}N) compared to the straw treatment without vinasses (1.7% of celery derived ^{15}N) probably due to an apparent added nitrogen interaction caused by displacement reactions with the soil microbial biomass. In conclusion, in raised beds, it is not possible to reduce nitrate leaching and to achieve a synchronization between the N release from crop residues and N demand by the following crop by using straw and vinasses to manipulate the N release from crop residues.

Keywords: ^{15}N-labelled celery residues; Straw; Vinasses; N immobilization; Priming effect

1. Introduction

Intensive field vegetable production is often characterized by an excessive use of organic N fertilizers and slurry, and large amounts of N-rich crop residues at harvest. Upon mineralization of these crop residues, mineral N amounts up to 150 kg N ha^{-1} can be released into soil (De Neve and Hofman, 1998). This often results in large nitrate contents in soil after harvest, and problems with respect to nitrate leaching. The vegetable sector throughout the European Community is under strong pressure to reduce its environmental impact, particularly with respect to nitrate leaching.

Since catch crops have a low N uptake efficiency when sown later then early September (Sörensen, 1992), they often give poor results in combination with vegetables harvested from September till December. Manipulating the N mineralization of N-rich crop residues may be an alternative method to reduce nitrate leaching in vegetable production. This implies the simultaneous mixing of crop residues with organic wastes with the intention that these organic wastes will either immobilize N released from crop residues or delay the N mineralization (i.e. *immobilizer wastes*). Characteristics like a high C:N ratio or high lignin content have proved to be indicators for a decreased N

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mineralization or N immobilization (Fox et al., 1990; Vigil and Kissel, 1991). In general, the application of organic materials with a C:N ratio higher than 20–40 (Fox et al., 1990; Vigil and Kissel, 1991) and/or a lignin content above 10% dry matter for legumes (Oglesby and Fownes, 1992; Constantinides and Fownes, 1994) and above 14% dry matter for non-legumes (Constantinides and Fownes, 1994) promotes net N immobilization or reduces the N mineralization. A high polyphenol content (>2–4% on dry matter) has shown to delay the N mineralization (Palm and Sanchez, 1991; Constantinides and Fownes, 1994; Bending et al., 1998). First, polyphenolic compounds are toxic for several micro-organisms, including bacteria, fungi and micro fauna involved in the process of N mineralization (Scalbert, 1991; Capasso et al., 1995; Hewlett et al., 1997). Secondly, polyphenols have a strong protein binding capacity through their strong affinity for amide groups. Therefore, the polyphenol effect on N availability is not an immobilization of mineralized N, but an inhibition of N mineralization through the above mentioned effects. Immobilizer wastes like straw, paper waste, green waste compost, saw dust and tannic acid (model compound for wastes with high polyphenol content) have been shown to possess a N immobilization potential (Rahn et al., 2003; De Neve et al., 2004; Chaves et al., 2005a, 2006). An additional beneficial effect would be achieved if the immobilized residue-N is remineralized by the time a new crop is sown or planted, induced by incorporating another organic waste (i.e. *remineralizer waste*), like molasses (De Neve et al., 2004) or vinasses (Chaves et al., 2005a). This remineralizer waste is expected to induce a priming effect, i.e. the N release after incorporation of this waste is higher than the sum of the N release from the remineralizer waste and the N release from the soil itself. Since it is mainly the recently immobilized N that can readily be remineralized (Jensen, 1994) a priming effect can be considered as remineralization of immobilized N. If the immobilized residue-N can be remineralized, the subsequent crop can benefit from the released N, and this could enhance the synchronization between the N release and the crop N demand, and so the N use efficiency.

In East-Flanders (Belgium) raised beds are regularly used in intensive field vegetable production, since soil temperature in these beds increases faster in spring, allowing an earlier planting of the crop. Also in other regions of the world raised beds are commonly used, for example in high-rainfall areas such as South Asia and Australia, since a better drainage in the beds makes the raised beds more water-efficient (Roth et al., 2005).

Incorporating ^{15}N-labelled crop residues in soil allows different N fractions to be followed (Jensen et al., 1997; Wivstad, 1999). Therefore, incorporating ^{15}N-labelled crop residues together with organic wastes under field conditions may reveal some of the mechanisms that take place in soil during the decomposition and may help to explain phenomena that may otherwise remain poorly understood.

The aim of this field study was to examine the effect of straw (as an immobilizer waste) and vinasses (as a remineralizer waste) on the N mineralization–immobilization turnover (NMIT) of ^{15}N-labelled crop residues when incorporated in raised beds. We hypothesized that a reduction of the celery derived ^{15}N in the soil mineral N pool after mixing the celery residues with straw would be the result of immobilization of celery derived mineral ^{15}N into a labile organic N pool, probably the microbial biomass, and that the addition of vinasses would lead to a remineralization of this immobilized celery-^{15}N.

2. Materials and methods

2.1. Crop residues and organic wastes

^{15}N-labelled celery residues (*Apium graveolens* L.) were chosen as crop residues because of their high N mineralization potential (De Neve and Hofman, 1996). The celery was grown in the greenhouse on a sandy soil which was poor in nutrients, in order to ensure that the celery would primarily use the added $K^{15}NO_3$ (20% abundance). Also a solution with P, K, Mg, S and Ca was given next to a solution of trace elements (Fe, Cu, B, Mn, Sn, Mo) to prevent nutrient deficiencies. The celery residues were separated into leaves and stems, dried at 55 °C and added in a known ratio (leaves:stems = 1:5 on dry matter) in order to achieve a homogeneous N mineralization and distribution of ^{15}N. Cereal straw was chosen as *immobilizer waste* since it has a high C:N ratio and was already shown to possess a N immobilization potential (Rahn et al., 2003; De Neve et al., 2004; Chaves et al., 2005a, 2006). Vinasses was chosen as *remineralizer waste*, because of its high content of easily decomposable C. It has been shown that addition of C in the form of sugars can lead to a marked increase in soil microbial activity (Falih and Wainwright, 1996). Vinasses is a final by-product of the sugar industry, and is the remaining material after fermenting molasses to alcohol, and removing the alcohol by distillation.

Subsamples (500 g fresh matter) of the celery residues and organic wastes were dried at 55 °C until constant weight for the determination of the dry matter content, and then milled (mesh size: 0.25 mm) for further analysis. Total C and N contents were determined on a 0.5 g dry subsample through a dry combustion under excess oxygen supply and high temperatures (850 °C) using a CNS elemental analyser (Variomax CNS, Elementar, Germany). The ^{15}N at.% of total N was determined on a dry subsample containing 100 μg N, using an elemental analyser (ANCA-SL, PDZ Europe) connected to an Isotope Ratio Mass Spectrometer (IRMS; Model 20–20, PDZ Europe). Lignin was determined by the Stevenson fractionation as modified by De Neve and Hofman (1996): (1) a 6 g dry subsample of the plant material was treated with 20 ml of 80% H_2SO_4 during 2.5 h, (2) after 2.5 h 200 ml distilled water was added and the mixture was

Table 1
Biochemical composition of celery residues, straw and vinasses

	DM (t ha^{-1})	N (kg ha^{-1})	DM (%)	N_{org} (g kg^{-1} DM)	^{15}N (atom%)	C:N	Lignin
Celery residues							
Leaves	1.59	71.0	17.8	27.2	8.02	10.0	24.7
Stems	2.35	59.0	9.07	22.5	9.27	14.2	25.8
Leaves + stems[a]	3.94	130.0	12.6	24.5	8.76	12.5	25.3
Immobilizer waste							
Cereal straw	12.0	52.7	86.2	4.4	–[b]	105.4	49.8
Remineralizer waste							
Vinasses	3.66	215.5	60.0	55.4	–[b]	7.38	–[c]

DM: applied dry matter; N: applied total N; DM: dry matter; N_{org}: organic N content.
[a] Sum/weighed average.
[b] Not applicable.
[c] No lignin fraction in vinasses.

boiled during 5 h under reflux, (3) lignin was the remaining fraction. The biochemical composition of the celery residues and organic wastes is presented in Table 1.

2.2. Experimental setup

The field experiment, a randomised complete block design with three replicates and plots of 1 m by 1 m, was set up on a loamy sand in the vegetable growing region in East-Flanders (Kruishoutem, Belgium). The field was laid out in raised beds of 25 cm height and 1 m wide (i.e. width of the plots). Some physical and chemical characteristics of the soil are presented in Table 2. The individual plots were separated by pathways of 1 m wide. Sampling was done while standing in the pathways in order to avoid compaction of soil under wet conditions.

The experiment lasted from late summer until the end of spring, and consisted of an immobilization and remineralization phase. At the start of the experiment (25 August 2004), each plot received dried ^{15}N-labelled celery residues (at a rate of 35 t FM ha^{-1}; ratio leaves:stems = 1:1.5 on dry matter) and straw, the immobilizer waste (equivalent to 5 t C ha^{-1}) which were incorporated with a rotavator to a depth of 25 cm (i.e. the height of a bed). The treatments were: (1) unamended soil, (2) celery only and (3) straw amended. Each treatment was replicated twice within each block during the immobilization phase, except the celery only treatment (see further), because the remineralizer waste was added to one of these two replicates per block at the start of the remineralization phase. Samples were taken with an auger (diameter 3 cm) to a depth of 75 cm in three layers: 0–25 cm, 25–50 cm and 50–75 cm. In each plot, four augerings were taken and the soil of duplicate treatments within one block was bulked into one composite sample per treatment per block. Samples were taken 13, 35, 62, 96, 133, 173 and 200 days after the incorporation of the organic materials.

At the start of the remineralization phase (14 March 2005; 200 days), one of the two replicates of the unamended soil and straw amended treatment within one block received the remineralizer waste vinasses (equivalent to 1.5 t C ha^{-1}) which was incorporated with a rotavator to a depth of 25 cm (i.e. the height of a bed). The celery only treatment, which was only needed to calculate the net N release from the celery residues, received no vinasses. This resulted in two additional treatments during the remineralization phase, namely (4) soil + vinasses and (5) straw amended + vinasses. During the remineralization phase, each treatment occurred once within each block. Four augerings were taken within each plot, and the samples were bulked into one composite sample per plot. Samples were taken 29, 58 and 92 days after the addition of vinasses.

2.3. Chemical analysis

Before analysing the soil samples, they were mixed very well and all visible celery and straw residues and other impurities were removed from the soil by hand. Total N and ^{15}N atom% were determined as for the celery residues, on dried (at 105 °C) and milled (mesh size 0.25 mm) subsamples containing 100 μg N. Mineral N (until a depth of 75 cm) in 30 g fresh soil samples was extracted with (1N) KCl (extraction ratio = 1:2) and the extracts were analysed for NH_4^+-N and NO_3^--N colorimetrically with a continuous

Table 2
Some physical and chemical properties of the loamy sand soil

Depth (cm)	Clay (0–2 μm) (%)	Silt (2–50 μm) (%)	Sand (50–2000 μm) (%)	OM (%)	BD (g cm^{-3})	pH_{KCl}
0–25	6.5	8.1	85.4	1.79	1.42	6.14
25–50	5.5	9.2	85.2	1.57	1.39	5.31
50–75	6.6	8.5	84.9	1.09	1.40	4.60

BD: bulk density; OM: organic matter.

flow auto-analyser (Chemlab System 4, Skalar, The Netherlands). The ^{15}N atom% of mineral N was determined on (1N) KCl extracts containing 10 μmol NO_3^- by converting NO_3^--N to N_2O-N (Stevens and Laughlin, 1994), and measuring ^{15}N atom% of N_2O using a trace gas module (ANCA-TGII, PDZ Europe) connected to the IRMS. Only the ^{15}N atom% of NO_3^- was determined since the NH_4^+ concentrations were very small compared to NO_3^-. Microbial biomass C was determined by fumigation–extraction according to Voroney et al. (1993) using a 24 h fumigation time, a 30 g fresh soil sample, a (0.1N) KCl extractant, a soil-to-extractant ratio of 1:2 (both for fumigated and non-fumigated samples) and a conversion factor k_{EC} of 0.25. Total organic C of the extracts was determined using a total organic carbon analyser (TOC-V_{CPN}, Shimadzu, Japan). Microbial biomass N was not determined directly, but was calculated from microbial biomass C, due to problems with the TN module of the total organic carbon analyser (TOC-V_{CPN}, Shimadzu, Japan), using a C:N ratio of 6.0, which can be considered as the average C:N ratio of microbial biomass (Powlson et al., 1987; Jensen et al., 1997). The use of a similar C:N ratio for the different treatments is supported by several studies that found no significant changes in C:N ratio of the microbial biomass after addition of organic materials (Bremer and van Kessel, 1992; Jensen et al., 1997).

2.4. Calculations

In the so-called 'difference method', the net N mineralization of the celery residues was calculated as the difference between the mineral N content in the celery residues only treatment (treatment 2) minus the mineral N content in the unamended soil (treatment 1). The N immobilization of straw was calculated as the difference between the mineral N content in the straw amended treatment (treatment 3) minus the mineral N content in the celery only treatment (treatment 2). The net N release from vinasses was calculated as the difference between the mineral N content in the soil + vinasses treatment (treatment 4) minus the mineral N content in the unamended soil (treatment 1). The priming effect (or remineralization of immobilized N) caused by vinasses was calculated as the mineral N content in the straw amended + vinasses treatment (treatment 5) minus the mineral N content in the straw amended treatment (treatment 3), minus the net N release of vinasses (treatment 4 minus treatment 1).With the ^{15}N method, the total ^{15}N recovery (%), the amount of celery derived ^{15}N in mineral N, the N immobilization of celery derived ^{15}N by straw and the remineralization of immobilized celery derived ^{15}N by vinasses were calculated as follows:

- Total ^{15}N recovery (%)

$$= \frac{\left(\frac{\text{at.\% }^{15}\text{Nexcess}_{\text{celery only or straw amended}}}{\text{at.\% }^{15}\text{Nexcess}_{\text{celery residues}}}\right) \times \text{total N}_{\text{celery only or straw amended}}\ (mg\,N\,kg^{-1})}{\text{total celery N added}\ (\text{mg N kg}^{-1})}$$

- Celery derived ^{15}N loading of the mineral N pool (mg N kg^{-1})

$$= \frac{\text{at.\% }^{15}\text{Nexcess}_{\text{celery only or straw amended}}}{\text{at.\% }^{15}\text{Nexcess}_{\text{celey only}}} \times \text{mineral N}_{\text{celery only or straw amended}}\ (\text{mg N kg}^{-1})$$

- % of celery derived ^{15}N in mineral N pool

$$= \frac{\text{amount of celery derived } ^{15}\text{N in mineral N}\ (\text{mg N kg}^{-1})}{\text{total celery N added}\ (\text{mg N kg}^{-1})}$$

- Immobilization of celery derived N by straw (mg N kg^{-1}) = amount of celery derived ^{15}N in mineral $N_{straw\ amended}$ (mg N kg^{-1}) − amount of celery derived ^{15}N in mineral $N_{celery\ only}$ (mg N kg^{-1})
- Remineralization of celery derived N by vinasses (mg N kg^{-1}) = amount of celery derived ^{15}N in mineral $N_{straw\ amended\ +\ vinasses}$ (mg N kg^{-1}) − amount of celery derived ^{15}N in mineral $N_{straw\ amended}$ (mg N kg^{-1})

Each time, corrections were made for natural abundance of ^{15}N in soil by subtracting the natural abundance of ^{15}N in unamended soil (0.3663 at.%) from the measured ^{15}N at.% in the celery amended treatments.

2.5. Meteorological data

The meteorological data were retrieved from the Provincial Experimental Station for Vegetable Production in Kruishoutem where the field trial was located (Fig. 1).

2.6. Statistical analysis

The measured values of mineral N in the 0–25 cm, 25–50 cm and 50–75 cm layer were analysed using repeated measures ANOVA (SPSS) with the sampling dates as the within effects. The presence of significant differences was tested using the pooled standard error of the difference (SED) and a Fisher's LSD at a 0.05 significance level. To determine whether the net N release of the celery residues, the N immobilization by the immobilizer wastes and the remineralization by the remineralizer wastes were significantly different from zero, one-sample *t*-tests (SPSS) were used.

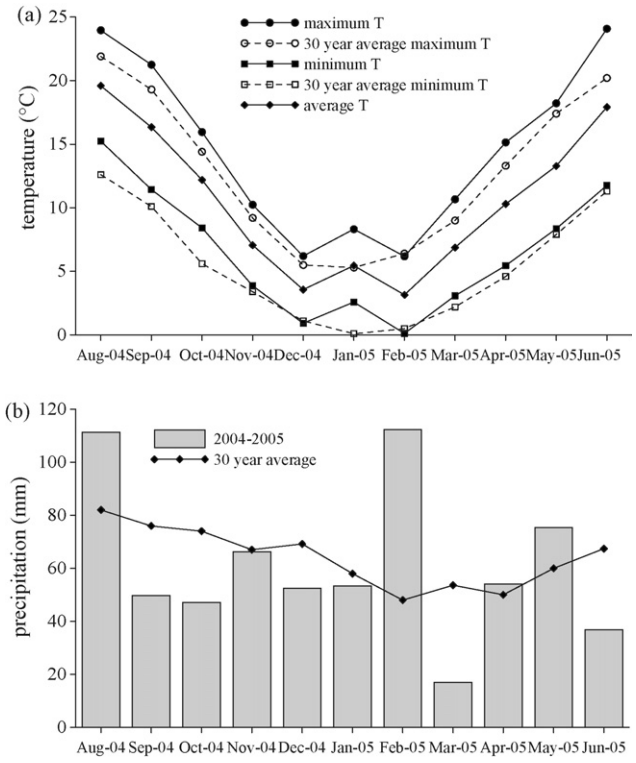

Fig. 1. Temperatures (a) and precipitation (b) during the field experiment.

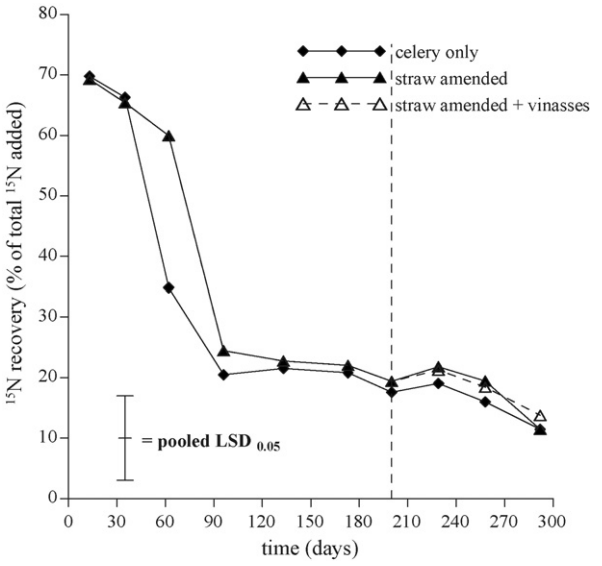

Fig. 2. Total ^{15}N recovery (% of total ^{15}N added) in top 25 cm in the celery only, straw amended and straw amended + vinasses treatment; error bar is pooled LSD at $P < 0.05$.

3. Results

3.1. Total soil N and total ^{15}N recovery

Total soil N was quite constant during the complete experiment, and as expected, no significant differences in total soil N content between the different treatments could be found. Total soil N was on average 4.88 ± 0.33 t N ha^{-1} for the top 25 cm. The total ^{15}N recovery of celery derived ^{15}N during the experiment is given in Fig. 2. At the first (13 days) and second (35 days) sampling date after the start of the experiment, the total ^{15}N recovery was 70% and 65% in the top 25 cm layer of both the celery only and straw amended treatment, respectively. After 35 days, the ^{15}N recovery significantly ($P < 0.05$) decreased in the celery only treatment, due to nitrate leaching, but not yet in the straw amended treatment. At day 62, the total ^{15}N recovery in the 0–75 cm layer was 51% in the celery only treatment and 76% in the straw amended treatment, of which 35% and 60% was recovered in the top 25 cm layer, respectively. The total celery-^{15}N losses from the 25 cm layer at day 62 corresponded to 85 kg N ha^{-1} and 52 kg N ha^{-1} for the celery only and straw amended treatment, respectively (Table 3). Hence, straw reduced the celery-^{15}N losses with 38% in the top 25 cm. From day 62, the total ^{15}N recovery also significantly ($P < 0.05$) decreased in the straw amended treatment. By the end of the immobilization phase (200 days), celery-N losses were similar in the celery only and in the straw amended treatment, namely 107 kg N ha^{-1} and 105 kg N ha^{-1} from the top 25 cm, respectively.

During the remineralization phase, no significant differences in ^{15}N recovery between the treatments could be found, and the additional celery-^{15}N losses were small (on average 8.5 kg N ha^{-1} for the three treatments). By the end of the experiment (day 292), the celery-^{15}N losses from the top 25 cm layer were on average 114.1 kg N ha^{-1} for all treatments.

3.2. ^{15}N recovery in mineral N (^{15}N method)

In the celery only treatment, the maximum amount of celery derived ^{15}N recovered in the mineral N pool was 44%, while at the same time the % of celery-^{15}N in the straw

Table 3
Losses of mineral ^{15}N (in kg N ha^{-1}) from the 0–25 cm and 0–75 cm layer

Time (days)	0–25 cm			0–75 cm		
	Celery only	Straw amended	Straw amended + vinasses	Celery only	Straw amended	Straw amended + vinasses
62	84.7 b	52.1 a	–[a]	63.5 b	31.0 a	–[a]
200	107.1 cd	104.8 c	–[a]	99.7 c	100.4 cd	–[a]
292	115.1 e	115.1 e	112.1 de	108.9 e	105.7 de	106.3 de

Different letters indicate significant differences for the 0–25 cm and 0–75 cm layer, separately (at $P < 0.05$).
[a] Not applicable.

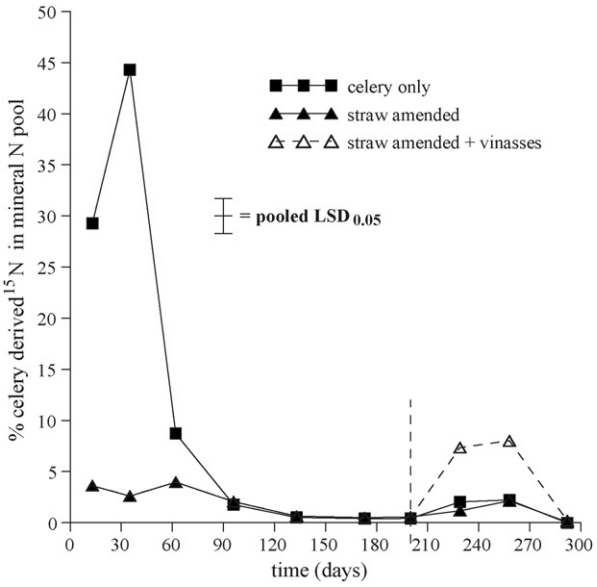

Fig. 3. Percentage of celery derived ^{15}N in the mineral N pool in the top 25 cm in the celery only, straw amended and straw amended + vinasses treatment; error bar is pooled LSD at $P < 0.05$.

amended treatment was only 3% (Fig. 3). Hence, straw immobilized almost all celery derived ^{15}N. After 35 days, the % of celery-^{15}N in the mineral N pool decreased and around 96 days almost no celery-^{15}N was found in the mineral pool in any of the treatments due to nitrate leaching.

After vinasses application, the percentage of celery-^{15}N significantly increased ($P < 0.05$) in the straw amended + vinasses treatment (7.6% of celery derived ^{15}N) compared to the celery only and straw amended treatment (2.1% and 1.7% of celery derived ^{15}N in the celery only and straw amended treatment, respectively; Fig. 3).

3.3. Mineral N (difference method)

During the immobilization phase, a significant ($P < 0.05$) redistribution was observed of mineral N to deeper soil layers and finally out of the profile, due to nitrate leaching (Fig. 4). After incorporation of the celery residues, the mineral N content significantly ($P < 0.05$) increased compared to the unamended soil, and the maximum net N release from the celery residues was 63.0 kg N ha^{-1} (48% of added N). In the straw amended treatment, the mineral N content did not significantly differ from the unamended soil, and the maximum N immobilization potential of straw was 66.9 kg N ha^{-1} (106% of released celery-N), indicating that straw immobilized all celery-N and even some soil-N.

In spring (after 200 days), the mineral N content significantly ($P < 0.05$) increased in all treatments and all soil layers as a result of higher temperatures and higher microbial activity (Fig. 4). When vinasses was added, the total mineral N content significantly ($P < 0.05$) increased compared to the treatments without vinasses. The increase in mineral N after vinasses addition was due to the net N

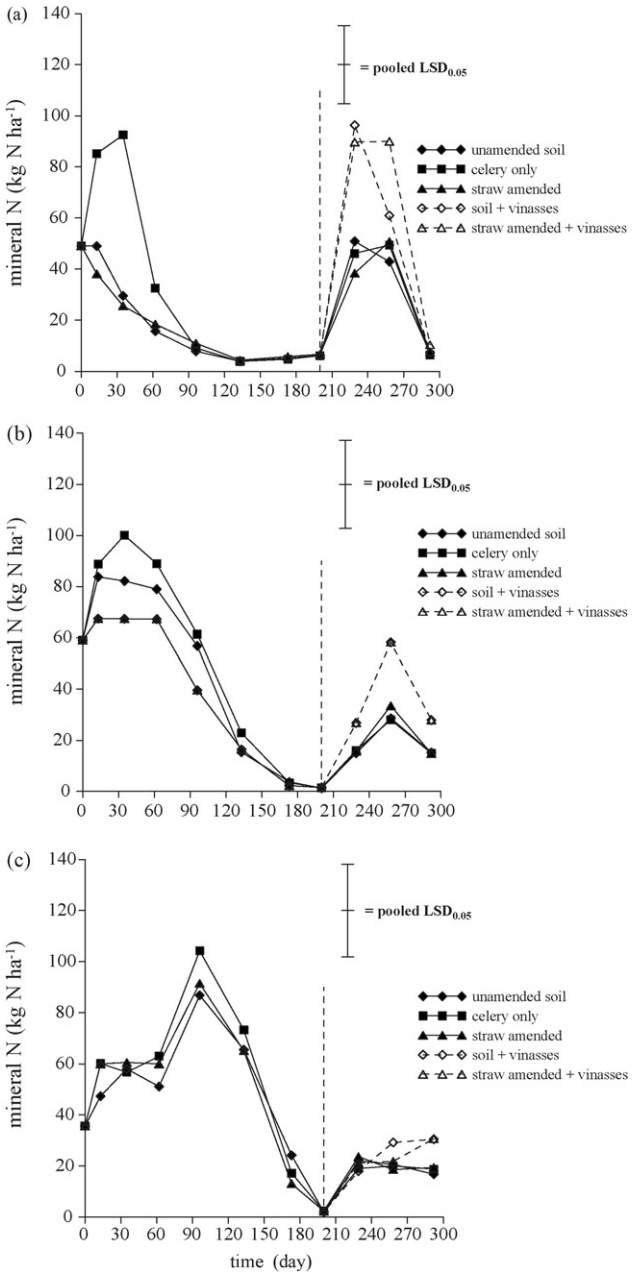

Fig. 4. Mineral N content in the different soil layers: (a) 0–25 cm, (b) 25–50 cm and (c) 50–75 cm; error bar is pooled LSD at $P < 0.05$.

release from vinasses itself (up to 45 kg N ha^{-1}) as was observed in the soil + vinasses treatment, and no extra N mineralization or real significant positive priming effect could be found in the top 25 cm.

3.4. Microbial biomass N (difference method)

Before incorporation of the celery residues and straw, the microbial biomass N accounted for 1.2% of total organic N in soil (55 kg N ha^{-1}) (Fig. 5). Similar values were found for sandy soils under continuous arable cropping (Powlson et al., 1987; Jensen, 1994; Jensen et al., 1997). The

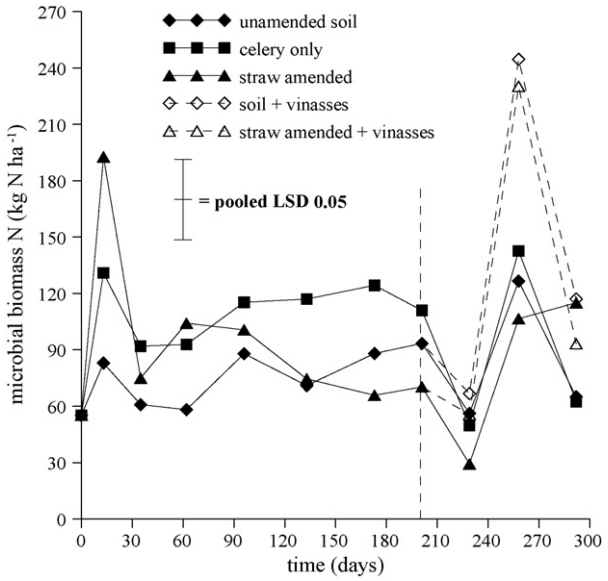

Fig. 5. Microbial biomass N in the top 25 cm in the different treatments; error bar is pooled LSD at $P < 0.05$.

incorporation of the ^{15}N-labelled celery residues led to a significant increase ($P < 0.05$) in microbial biomass N which peaked after 13 days and was 48 kg N ha^{-1} (37% of added celery-N) higher than in the unamended soil. Mixing the ^{15}N-labelled celery residues with straw led to an extra increase in microbial biomass N compared to the celery only treatment at day 13 (significant at $P < 0.05$). Maximum microbial biomass N in the straw amended treatment was 110 kg N ha^{-1} larger compared to the unamended soil or 60% of total added N (= celery + straw-N). From day 35, microbial biomass N, both in the celery only treatment and in the straw amended treatment, decreased and became similar as in the unamended soil, and no significant differences could be found between the treatments during the rest of the immobilization phase.

When vinasses was added after 200 days, microbial biomass N significantly ($P < 0.05$) increased compared to the treatments without vinasses (Fig. 5).

4. Discussion

4.1. Difference method and ^{15}N method

The main drawback of the difference method compared to the ^{15}N method is that it assumes that the basal N mineralization is the same in the presence and absence of residues (Watkins and Barraclough, 1996). Several studies have confirmed that this assumption is not always correct and that the N release from soil organic matter may increase or decrease after incorporation of fresh organic matter (Cadisch et al., 1998; Wivstad, 1999; Kuzyakov et al., 2000). However, in this study, the amount of N released from the labelled celery residues did not differ significantly between both calculation methods (48% in difference method, 44% in ^{15}N method), indicating that the incorporation of celery residues did not affect the N release from the soil organic matter. This may also indicate that the basal microbial biomass was not influenced by the incorporation of celery residues, and that the increase in microbial biomass N in the celery only treatment was solely due to the immobilization of celery-N into the microbial biomass.

Another difference between the two calculation methods, especially when examining the N immobilization by straw and the remineralization by vinasses, is that the difference method does not distinguish between the immobilization or remineralization of soil-N and celery-N, while the ^{15}N method only gives the immobilization or remineralization of the celery derived ^{15}N. Straw can immobilize both soil-N and celery-N, which cannot be derived from the results of the ^{15}N method. Hence, the ^{15}N method may underestimate the N immobilization potential of straw.

Also the remineralization of immobilized N by vinasses was quite different between the two calculation methods. A priming effect occurs when the N release is higher than the sum of the N release from the added material and the N release from the soil, meaning that an extra amount of mineral N is released from soil organic matter which can be both native soil organic N or recently immobilized celery-N (Jenkinson et al., 1985; Powlson and Barraclough, 1993). By using the difference method a real and complete priming effect was calculated (i.e. the release of both soil-N as celery-N), while the ^{15}N method only indicated a partial the remineralization of celery derived ^{15}N. Hence, the difference method gives a better idea of the total amount of mineral N available for the subsequent crop, while the ^{15}N method only indicates whether the immobilized celery-N is remineralized or not. Both calculation methods should therefore be used complementary, but from practical point of view (N availability) the difference method gives more relevant information, e.g. for the farmer.

4.2. N release from celery residues

During the first 35 days after the incorporation, the celery residues released already 48% of their N-content as mineral N, which was accompanied by high microbial activity. During the first 62 days of the experiment, air temperatures were high (on average 14.9 °C), and since soil in raised beds is especially sensitive to changes in air temperature, a microclimate was created in the beds leading to high microbial activity and high N mineralization. The sandy texture of the soil also led to favourable conditions for N mineralization, e.g. a more favourable pore size distribution, gas exchange, mobility of cells and diffusion of substrates, as compared to fine-textured soils (Schjonning et al., 1999). The use of dried celery residues also added to the high N mineralization, especially since the celery residues were easily pulverised due to the incorporation with a rotavator. Dried and pulverised residue material is more accessible to

micro-organisms than intact plant parts, due to the increased surface area of the residues exposed to decomposition (Angers and Recous, 1997) and the lack of intact lignified barrier tissue (Summerell and Burgess, 1989). Therefore, the initial colonization rate of the residues, and N mineralization was favoured. When fresh residues are added to the soil, the N mineralization will be slower, but the final amount of N released from the residues will be similar as from dried residues. In an incubation study, fresh celery residues released 50% of their total N after 45 days (Chaves et al., 2005a). In a field study, fresh cauliflower residues released 41% after 42 days (Chaves et al., in press).

The large mineral ^{15}N losses from the top 75 cm soil layer (Table 3) were due to heavy rainfall during the first days after the start of the experiment (34.4 mm) and the sandy texture of the soil, what led to nitrate leaching and to gaseous N losses. Chaves et al. (2005b) proved that denitrification directly after incorporation of dried celery residues can be very high. The total mineral N losses from the soil will have been even larger since also unlabelled soil-N was lost from the soil layer by nitrate leaching or denitrification.

4.3. N immobilization by straw

A straw amendment of on average 5 t C ha^{-1} has shown to be a suitable method to reduce nitrate leaching after incorporation of crop residues, both in laboratory experiments (Rahn et al., 2003; De Neve et al., 2004; Chaves et al., 2005a, 2006) as field experiments (Chaves et al., in press). Also in this study, mixing straw with celery residues led to a significant increase in microbial biomass and a significant decrease in mineral celery-^{15}N during the first 62 days, indicating that the celery-N was immobilized in microbial biomass N where it was protected from leaching. Straw indeed reduced the celery-N losses during the first 62 days after the incorporation, but could not conserve the celery-N in soil until the next spring (after 200 days). This was probably due to a relatively short period of N immobilization by straw followed by a natural remineralization of celery-^{15}N (without addition of vinasses) when the risk of nitrate leaching and denitrification was still high. A reason for natural remineralization of celery-^{15}N (without addition of vinasses) could be a decrease in air temperature from on average 14.9 °C during the first 62 days to air temperatures frequently below 10 °C thereafter (on average 4.8 °C between day 62 and day 200). Since soil in raised beds is especially sensitive to changes in air temperature, soil temperatures must have decreased substantially. Due to the low soil temperatures, micro-organisms died, leading to the release of microbial biomass N. It has been found that gross N immobilization is more sensitive to low temperatures than gross N mineralization (Andersen and Jensen, 2001) probably due to a lower affinity of micro-organisms to both organic and inorganic substrate (Nedwell, 1999), leading to a net N release at low temperatures. In fields without raised beds, soil temperatures are less sensitive to fluctuating air temperatures and do not decrease as fast as in field with raised beds, what explains that a straw amendment in fields without raised beds can be a good management option to reduce nitrate leaching (Chaves et al., in press).

4.4. Remineralization

In the straw + vinasses treatment, vinasses was added at the start of the remineralization phase (day 200), with a view to enhance remineralization of immobilized celery-N (i.e. priming effect). Although it is widely accepted that micro-organisms play a crucial role in the process, the mechanisms leading to priming effects remain poorly understood. The most common explanation for a priming effect is an increased soil organic matter decomposition as a result of a higher microbial population or activity due to the higher availability of energy and nutrients from added organic materials (Kuzyakov et al., 2000; Fontaine et al., 2003).

According to the ^{15}N method, vinasses stimulated the remineralization of immobilized celery-^{15}N, while the difference method showed that vinasses was not able to cause a real positive priming effect. This remineralization of celery-^{15}N found by the ^{15}N method could be due to an apparent added nitrogen interaction caused by displacement reactions with the soil microbial biomass rather than a real priming effect (Jenkinson et al., 1985; Chaves et al., 2006). After application of vinasses-N to soil, immobilization–mineralization reactions incorporate the added vinasses-N into the biomass with the release of microbial biomass ^{15}N into the inorganic pool, leading to a higher release of mineral ^{15}N in the straw + vinasses treatment than in the straw treatment where no vinasses-N was added.

Also in most other studies, it has been shown that remineralization of immobilized is difficult to obtain (Chaves et al., 2005a, 2006, in press). An explanation for the low remineralization induced by vinasses could be the low amount of immobilized-N available by the time of addition of vinasses since it is mainly recently immobilized N that can be readily remineralized (Jensen, 1994). Another possible reason for the low remineralization potential of vinasses may be its unsuitable biochemical composition, e.g. high molecular weight and not readily decomposable, for inducing N priming effects, since most researchers found N priming effects after incorporation of low molecular weight compounds like glucose (Falih and Wainwright, 1996; Wheatly et al., 2001).

5. Conclusions

Raised beds have a significant influence on the N mineralization–immobilization turnover in soil due to their sensitivity to changes in air temperatures. In late summer, higher soil temperatures in raised beds lead to a large N mineralization from crop residues and large N immobilization by straw, as compared to fields without raised beds.

However, in autumn and winter, low soil temperatures in raised beds shorten or prevent N immobilization by straw and lead to natural remineralization of immobilized N (without addition of vinasses) when the risk of nitrate leaching is still high. This makes it impossible to retain crop residue-N in soil until the following spring, and shows that, in raised beds, a straw amendment is not a suitable method to reduce nitrate leaching. In fields without raised beds, a straw amendment of on average 5 t C ha^{-1} has shown to be a good management option to reduce nitrate leaching. In both fields with and without raised beds, remineralization of immobilized N is difficult to obtain in a consistent manner. Hence, in raised beds, it seems to be difficult to reduce nitrate leaching after incorporation of crop residues and to achieve a synchronization between crop residue-N release crop N demand, by using straw and vinasses.

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